DEVELOPMENT OF THE FEMALE GAMETOPHYTE IN ERYTHRONIUM HELENAE AND ERYTHRONIUM TUOLUMNENSE

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The type of development of the female gametophyte which Bambacioni (2) described for *Fritillaria* in 1928 has been shown to occur in the first five of the ten genera listed by Hutchinson (10) in the tribe Tulipeae: *Erythronium, Fritillaria, Tulipa, Gagea, Lilium, Lloydia, Nomocharis, Notholirion, Giraldiella,* and *Calochortus.* In this type of development, of the four nuclei resulting from the two divisions of meiosis, three migrate to the chalazal end of the embryo sac while the fourth remains at the micropylar end. At the following division the latter divides normally giving rise to two haploid nuclei. The chromosomes of the three dividing chalazal nuclei become aligned on one spindle, thus giving rise to two daughter nuclei, each of which has 3n chromosomes. This second group of four nuclei goes through the final division to produce an eight-nucleate gametophyte with four haploid nuclei at the micropylar end and four triploid at the chalazal. The primary endosperm nucleus is formed by fusion of a polar nucleus from each group and is thus 4n.

Representatives of the five genera in which the *Fritillaria* type of female gametophyte development is known are: *Fritillaria persica* (2); *Tulipa Gesneriana* (3), and a triploid form of the same (4); *Erythronium Dens-canis* (9), and *E. japonicum* (13); *Lilium Henryi* (6), *L. philippinense* (15), *L. tigrinum* (20), and *Cardiocrinum cordatum* Makino (= *Lilium cordifolium* Thunberg) (12); *Gagea minima* and *G. lutea* (11, 19), *G. ova, G. graminifolia* and *G. tenera* (14).

So far as the author knows no work has yet been published on *Lloydia, Giraldiella, Notholirion* and *Nomocharis. Calochortus* (5) is the only genus of the tribe thus far shown to have the "normal" type of macrosporogenesis and development of the female gametophyte.

In *Erythronium* both the *Fritillaria* and *Adoxa* types of development have been described. Hrubý (9) described the process in *Erythronium Dens-canis* with twelve pairs of chromosomes up to the stage where three nuclei are seen at the chalazal end and it may be assumed that this species would hold to the *Fritillaria* type. Oikawa (13) has followed the complete development in *Erythronium japonicum* (n = 12) and found it to be of the *Fritillaria* type. However, Cooper (7) has found the *Adoxa* type in a 22-paired *Erythronium albidum.* Schaffner (17) described gametophyte development for *Erythronium americanum* and *E. albidum* (both with twelve pairs of chromosomes). From his description and figures development seems to follow the *Adoxa* scheme, but

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since this work was done prior to 1928 there may be a possibility that Schaffner missed seeing the chalazal fusion as did all other workers before this time. Guerin (8) stated that the development in *Erythronium Dens-canis* is analagous to that which has been known "depuis longtemps chez *Lilium, Tulipa,* et *Fritillaria.*"

According to Applegate (1) there are three to four species of *Erythronium* in Eurasia, five in North America east of the Rockies, and fifteen west of the Rockies. Since those of eastern North America and Europe and Asia apparently show differences in the type of development of the female gametophyte, it was thought that study of this point in some of the western North American species would be of interest. Investigation of two Californian species, *Erythronium helenae* Applegate of section Concolorae and *E. tuolumnense* Applegate of section Pardalinae was therefore undertaken.

Materials. Ovaries of these two species of *Erythronium* were fixed in CRAF, dehydrated by normal butyl alcohol, and embedded in paraffin. Sections were cut at 10, 15, and 20 microns. Three stains were employed: Heidenhain's iron alum haematoxylin, Stockwell's modification of Fleming's triple stain, and the Feulgen stain, counterstained with fast green. Haematoxylin was perhaps the best for sections at 10 microns but the Feulgen and fast green was by far the best for thicker sections.

Development of the Female Gametophyte

The embryo sac mother cell (the archesporial cell) is located directly below a single layer of nucellus cells (pl. 20, figs. 1 and 2). The cytoplasm is finely vacuolate throughout with a somewhat denser layer around the spindle. At metaphase of the heterotypic division (pl. 20, fig. 3) it was impossible to determine the exact number of bivalents since all metaphase plates seen were in more than one section and some of the bivalents may have been cut. However, there seemed to be around twelve in *E. helenae.* No heterotypic mitoses were observed in *E. tuolumnense,* but somatic plates indicated no large number such as Cooper (7) found in *E. albidum.*

The two nuclei resulting from the heterotypic division lie at opposite ends of the embryo sac (pl. 21, fig. 4). There is no resting period at this time and the homeotypic division proceeds at once. The spindles of this division also are surrounded by a dense layer of cytoplasm (pl. 21, figs. 5 and 6). No vacuole is yet observable in the sac.

At the end of this division there are four haploid nuclei in the sac. In *Erythronium helenae,* but not in *E. tuolumnense,* faint lines which are apparently fibers are often seen connecting the nuclei. Text figure 1 which is a camera lucida drawing of figure 8 (pl. 22) shows that fibers connect the two central nuclei not only with each other but also with the nuclei at the micropylar and chalazal ends. Stenar's (18) figures 3b and 7c show connecting fibers in

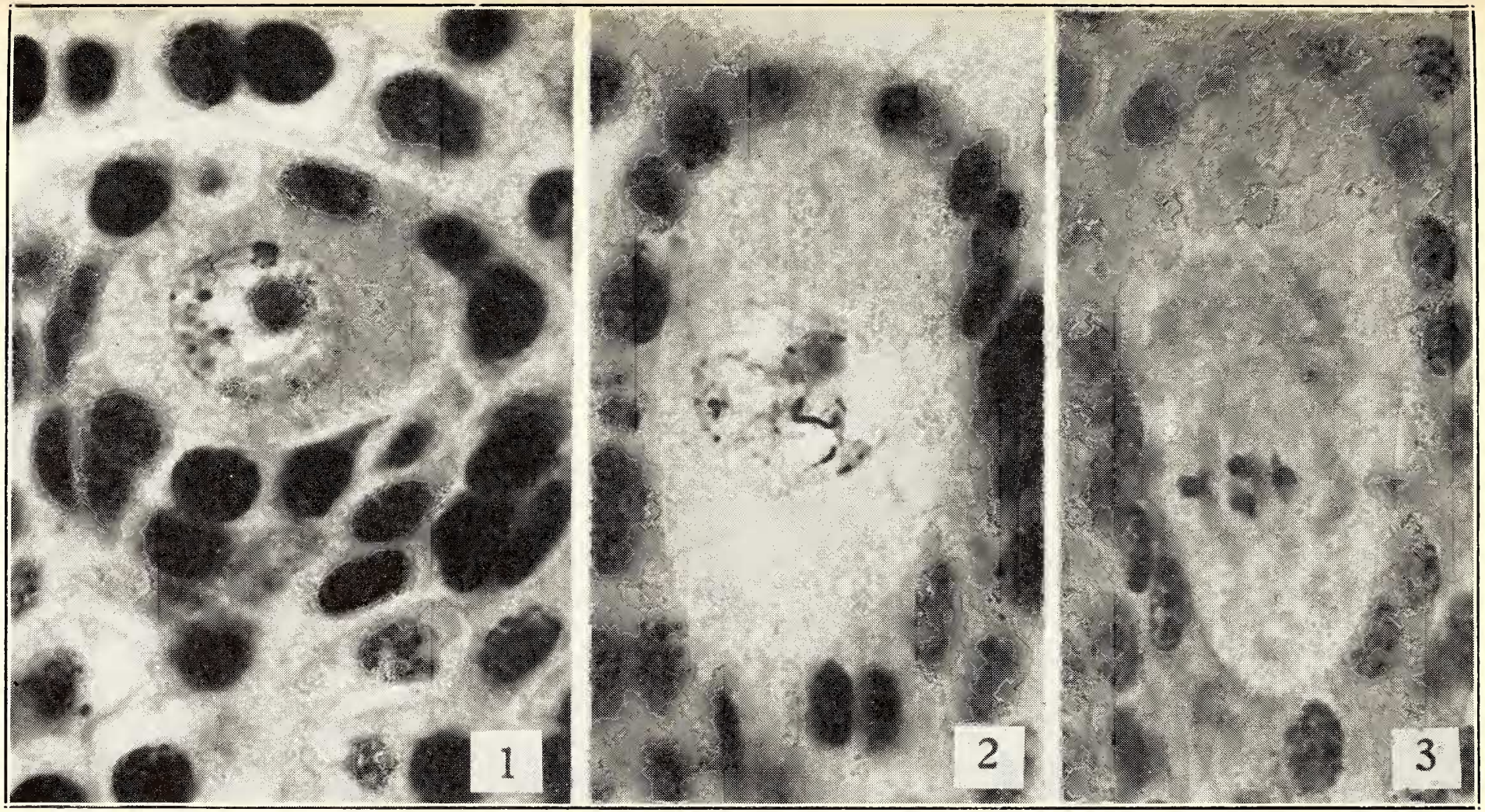

PLATE 20. DEVELOPMENT OF FEMALE GAMETOPHYTE IN ERYTHRONIUM. Fig. 1. Archesporial or embryo sac mother cell in *E. helenae*. Fig. 2. Prophase of heterotypic division in *E. tuolumnense*. Fig. 3. Metaphase of heterotypic division in *E. helenae*. All × 700.

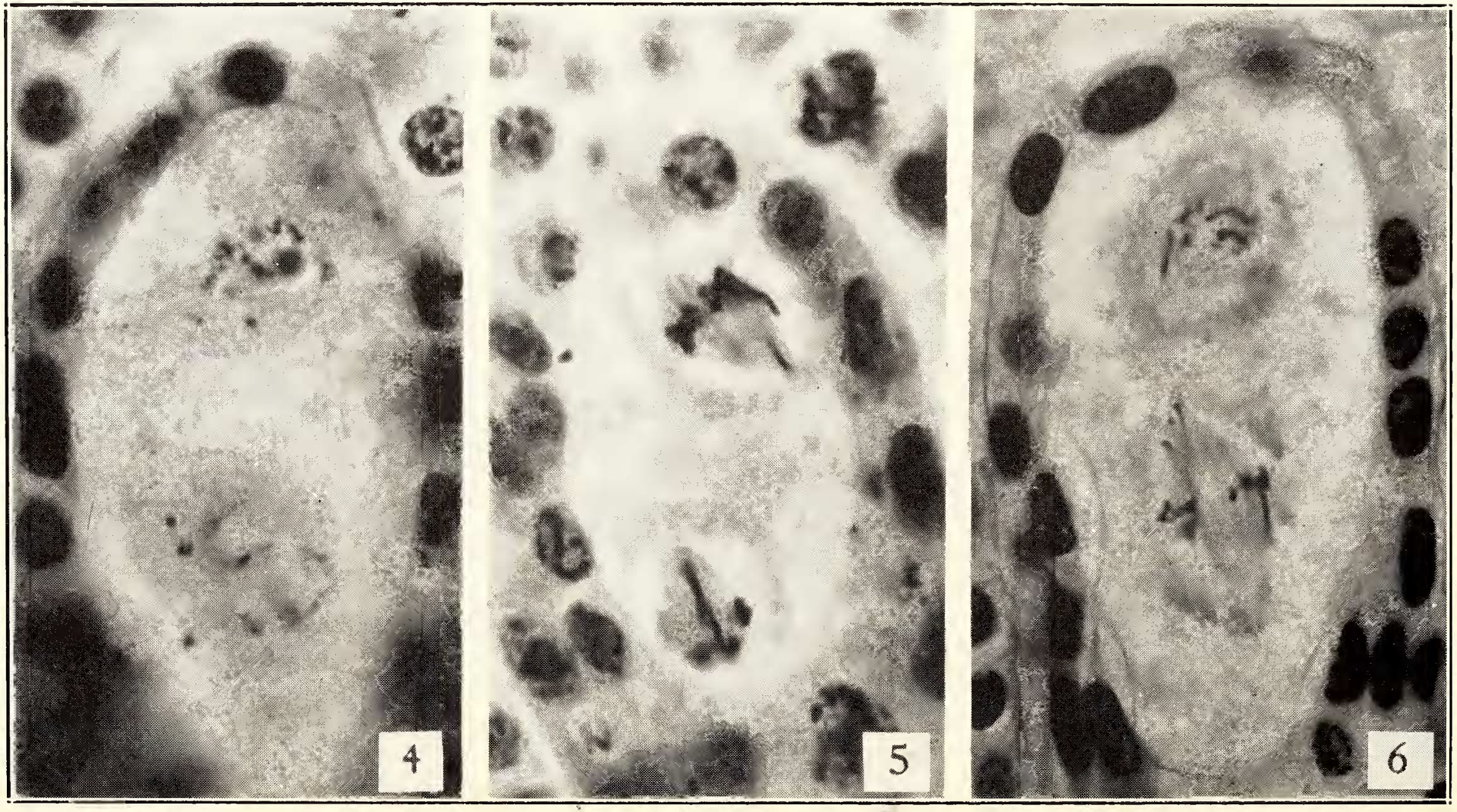

PLATE 21. DEVELOPMENT OF FEMALE GAMETOPHYTE IN ERYTHRONIUM. Fig. 4. Two nuclei resulting from heterotypic division in *E. helenae*. Fig. 5. Prophase of second division in *E. tuolumnense*. Fig. 6. Prophase of second division in *E. helenae*. Note dense cytoplasmic region around spindles. All × 700.

Gagea lutea and *G. minima* in the same configuration. Figure 7 (pl. 22) shows three nuclei at the micropylar end connected by fibers and the lowest of these furthermore connected with the nucleus at the chalazal end (in the adjacent section). Bambacioni and Giombini's (3) figure 9 in *Tulipa Gesneriana* shows a similar arrangement of fibers. In Romanov's (14) photomicrographs of *Gagea graminifolia* in his figure 7c the three chalazal nuclei can be seen connected by faint fibers. Joshi (11) mentions secondary spindle fibers arising to connect the homeotypic spindles in *Gagea fascicularis*.

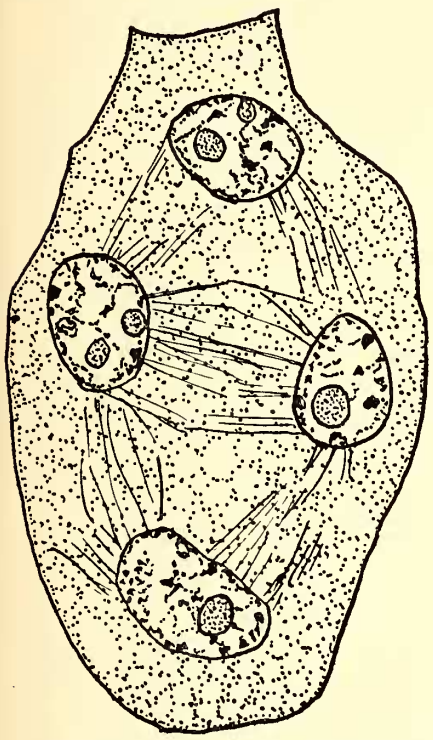

Fig. 1. Four haploid nuclei in *Erythronium helenae* all connected by fibers (camera lucida drawing of embryo sac shown in pl. 22, fig. 8).

Since these four haploid nuclei are the result of meiosis and therefore correspond to the four macrospores of a "normal" macrosporogenesis which are separated by cell walls, it may be that the fibers under discussion represent vestigial traces of cell wall initiation between the spores. If walls should form across all the fibers in figures 7 and 8 (pl. 22) each nucleus would then be separated from every other as in spore formation. A somewhat similar condition exists in a young multicellular endosperm where fibers arise to connect all the nuclei, and walls separating them then appear across the fibers throughout the endosperm. However, no walls are produced between the haploid nuclei in the embryo sac and the fibers have completely disappeared by the beginning of third prophase.

In both species three of the four haploid nuclei migrate to the chalazal end of the sac. In *E. tuolumnense* a large vacuole separates the three chalazal nuclei from the micropylar and a smaller vacuole is found at the center of the three (pl. 22, fig. 9). In *E. helenae* no vacuole is present until the second four-nucleate stage (pl. 23, fig. 12 and pl. 24, fig. 14).

At prophase of the third division the three chalazal nuclei start to fuse (pl. 23, figs. 10 and 11). Often from telophase of the second division through metaphase and telophase of the third in *E. helenae* many small bodies in the cytoplasm which stain black with haematoxylin, but do not stain with the Feulgen technique, are seen (pl. 23, fig. 12). They are pictured by Schaffner (17) in *E. albidum* in his figure 59 although this is apparently only the telophase of the first division. Hrubý shows them at the telophase of the second division and suggests that they are leuco-